

FIG. 1

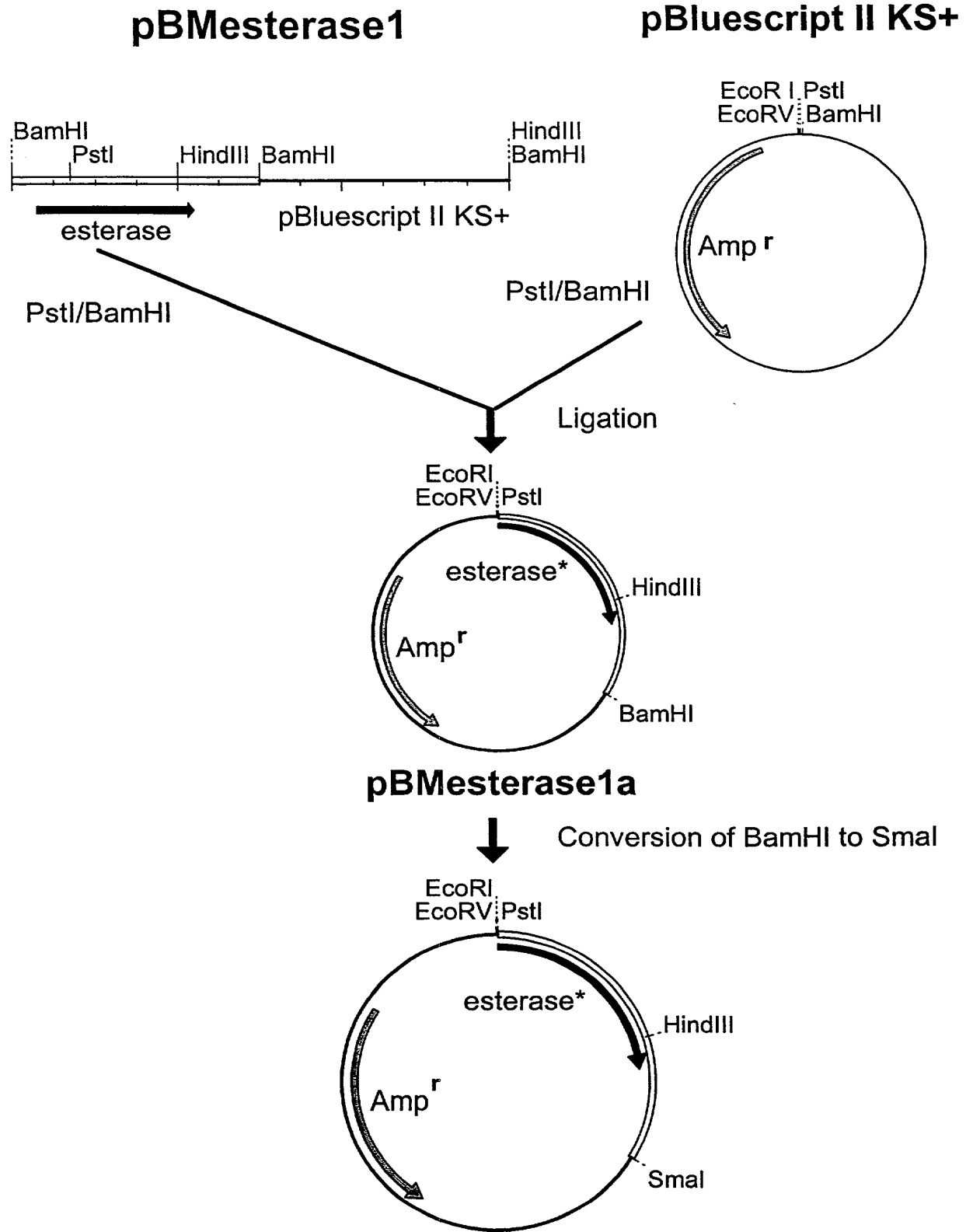


FIG. 2

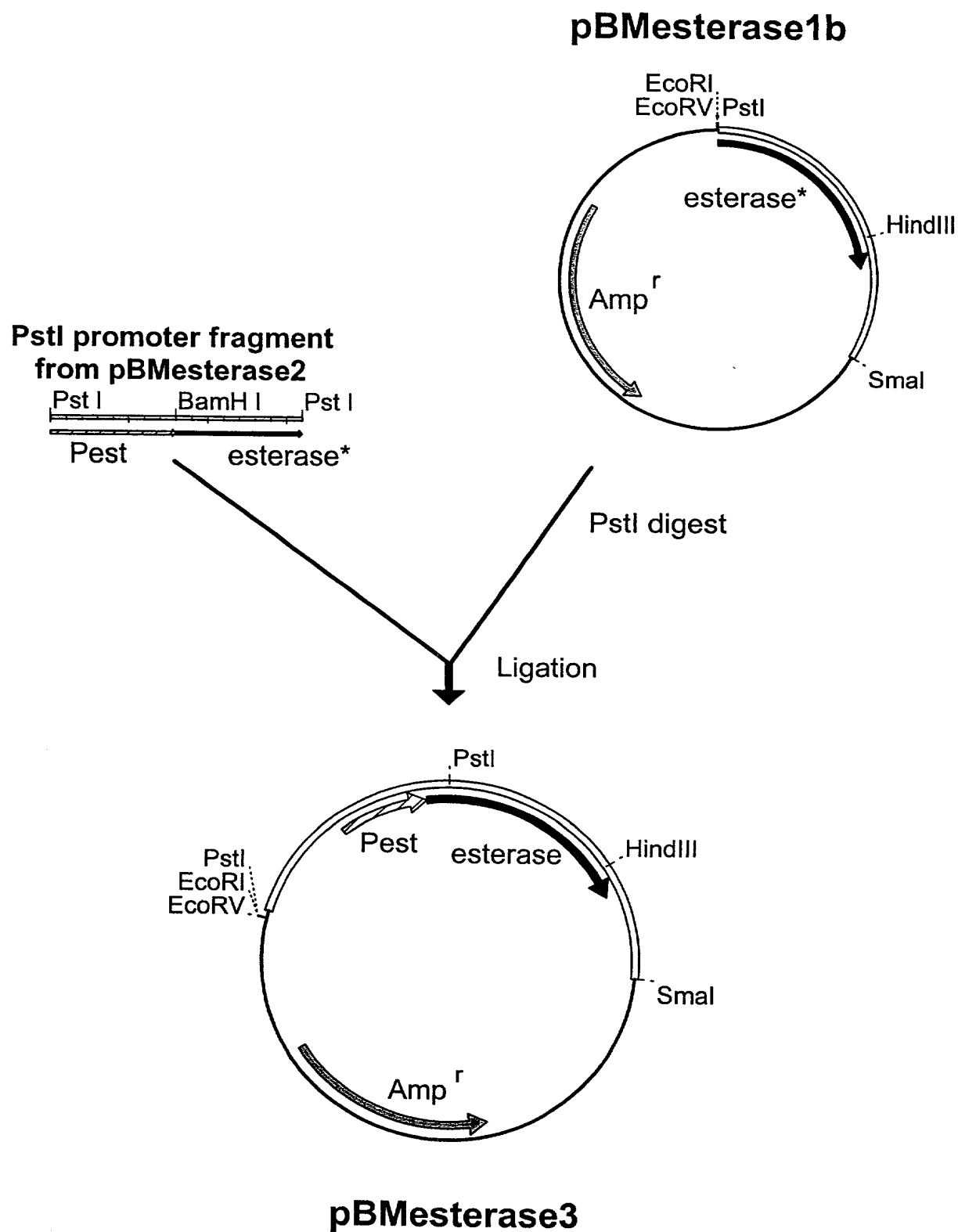
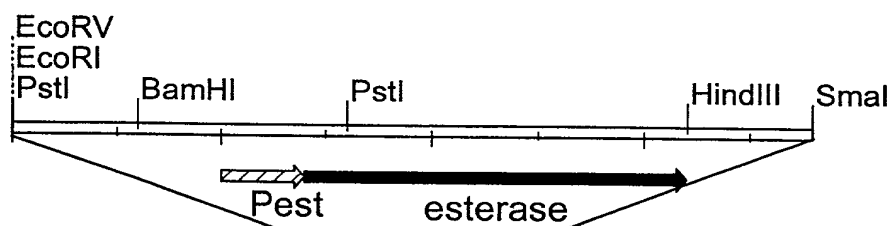


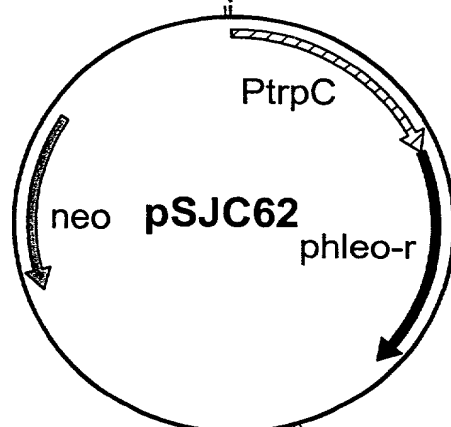
FIG. 3

SmaI/ EcoRV esterase fragment from pBMesterase3

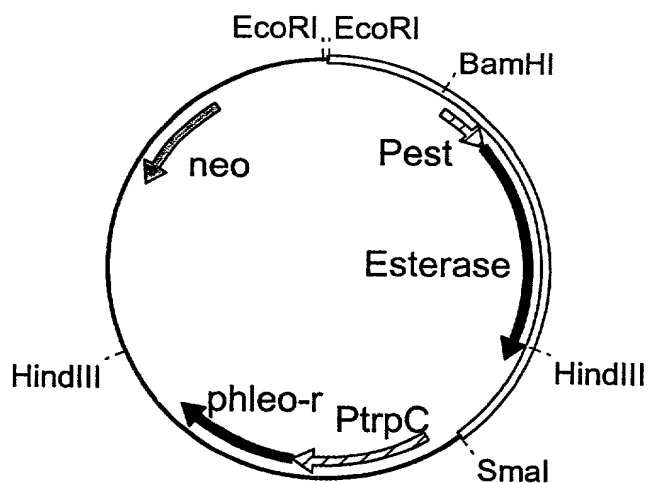


BamHI
SmaI
EcoRI

- 3.8 kb EcoRV/SmaI fragment inserted into SmaI site of pSJC62.



XbaI
HindIII



pSJC62.3

FIG. 4

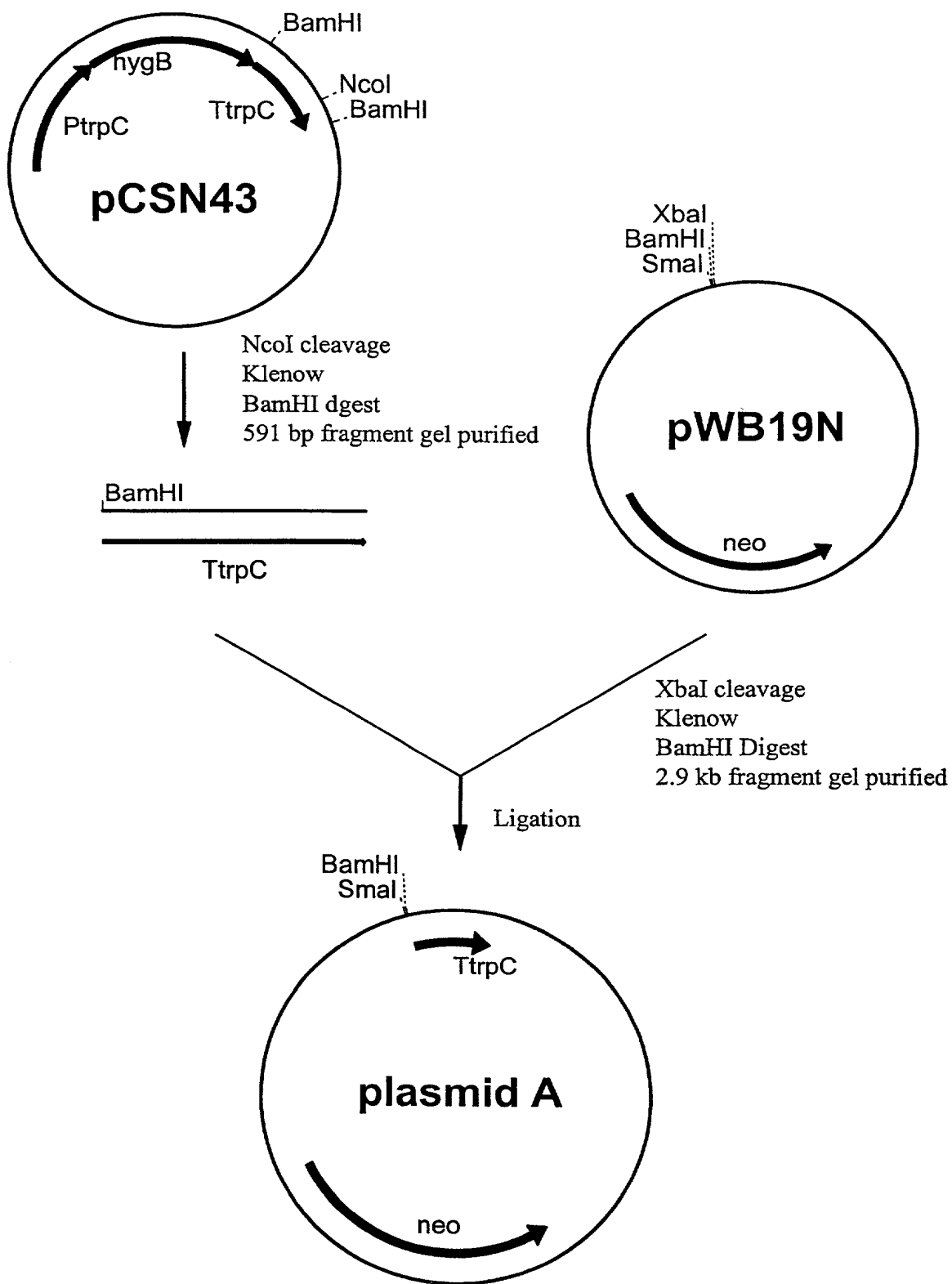


FIG. 5

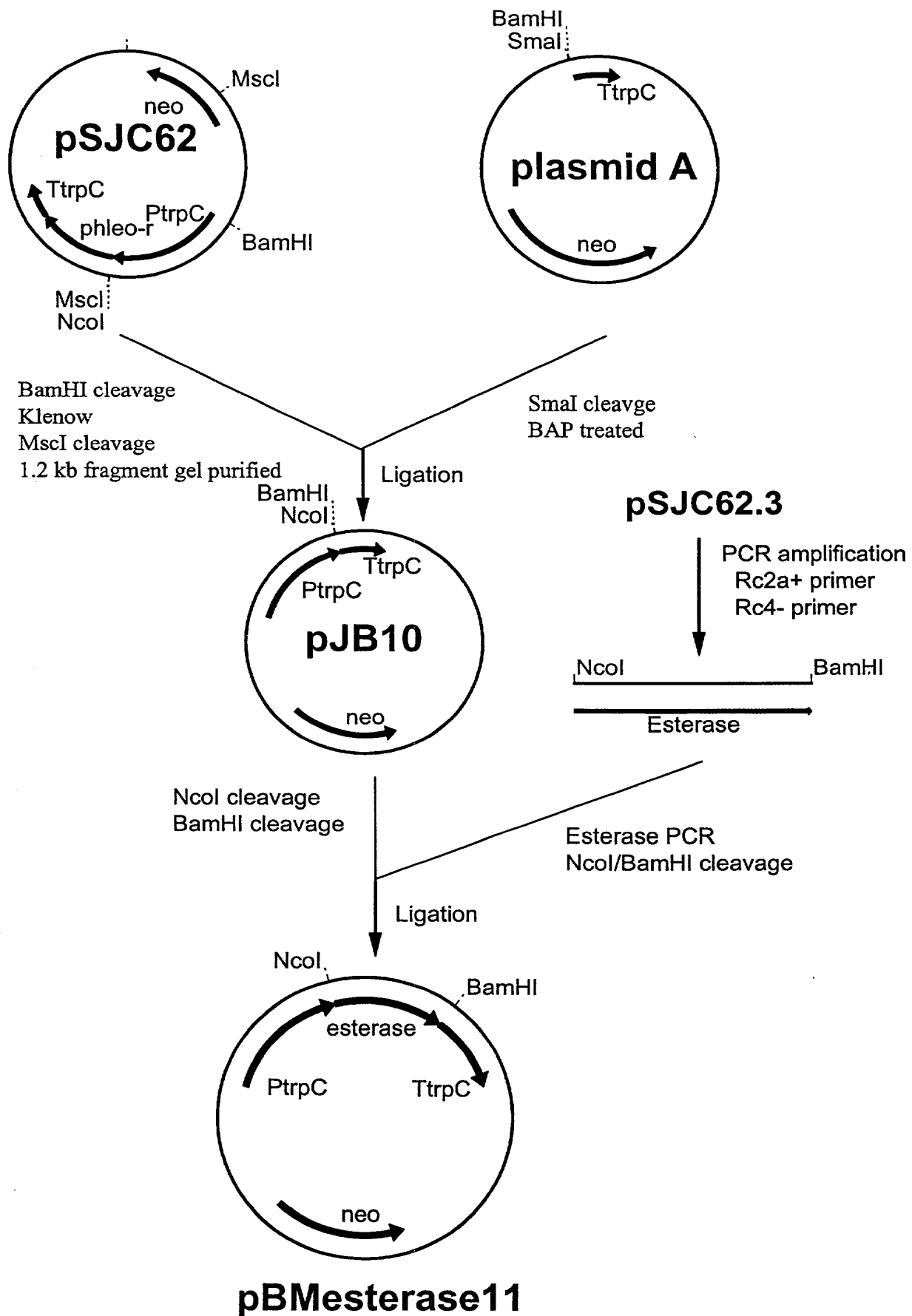


FIG. 6

N-TERMINAL AMINO ACID SEQUENCE

AMINO ACID SEQ.		T	N	P	N	E	P
REV. TRANSLATION		ACX	AAPy	CCX	AAPy	GAPu	CC
INVERSE		GGPy	TCPu	TTX	GGPu	TTX	GT
PROBE	1	GGPy	TCPu	TTG	GGPu	TTX	GT
	2			A			
	3			T			
	4			C			

Four 17-mer oligonucleotide probes each with a 32-fold degeneracy were synthesized from the N-terminal amino acid sequence and used to probe a Southern blot of *R.toruloides* DNA.

FIG. 7

RHODOSPORIDIUM ESTERASE cDNA

ATGCTCCTTAACCTCTTCACCCCTCGCCTCCCTCGCTGCGACGCTCCAGCTCGCCTTTGCC	70
M L L N L F T L A S L A A T L Q L A F A	
TCTCCGACCTCCCTCGTCCGCCGCACGAACCCAAACGAGCCCCCTCCCGTCGTCGACCTC	130
S P T S L V R R T N P N E P P P V V D L	
GGCTACGCCCCGCTACCAAGGCTACTTGAACGAGACCGCCGGACTCTACTGGTGGCGCGGA	190
G Y A R Y Q G Y L N E T A G L Y W W R G	
ATCCGCTACGCCTCGGCTCAGCGCTTCCAGGCTCCTCAGACGCCCGCGACGCACAAGGCC	250
I R Y A S A Q R F Q A P Q T P A T H K A	
GTCCGCAACGCGACTGAGTATGGACCGATCTGTTGGCCGGCTAGCGAGGGAACCAACACG	310
V R N A T E Y G P I C W P A S E G T N T	
ACCAAGGGCTTGCCGCCGCCTAGCAACAGCTCGAGCAGCGCGCCGCAGAAACAGGCGTCG	370
T K G L P P P S N S S S S A P Q K Q A S	
GAGGATTGCCTCTTCCTCAATGTCGTTGCCCCGCGGCTCGTGCGAGGGCGACAATCTT	430
E D C L F L N V V A P A G S C E G D N L	
CCCGTCTCGTCTACATTCACGGAGGTGGCTACGCCTTCGGCGATGCGAGCACCGGCAGC	490
P V L V Y I H G G G Y A F G D A S T G S	
GACTTTGCCGCTTCACCAAGCACACGGGAACCAAGATGGTCGTTGTAATCTCCAGTAC	550
D F A A F T K H T G T K M V V V N L Q Y	
CGTCTCGGCAGCTTTGGTTTCCTCGCTGGCCAAGCCATGAAGGACTACGGTGTAACGAAC	610
R L G S F G F L A G Q A M K D Y G V T N	
GCCGGCTTGCTTGACCAGCAATTCGCCCTTCAATGGGTTCAACAGCACGTCTCGAAGTTC	670
A G L L D Q Q F A L Q W V Q Q H V S K F	
GGCGGCAACCCCGATCACGTTACGATTTGGGGCGAGTCTGCAGGCGCAGGGTCCGTTATG	730
G G N P D H V T I W G E S A G A G S V M	
AACCAGATCATTGCGAACGGCGGCAACACCGTCAAGGCTCTCGGTCTCAAGAAGCCCCCTC	790
N Q I I A N G G N T V K A L G L K K P L	
TTCCACGCTGCCATCGGCTCCTCCGTCTTCTCCCTACCAAGCCAAGTACAACTCCCCC	850
F H A A I G S S V F L P Y Q A K Y N S P	
TCGCGGAGCTGCTCTACTCCCAACTCGTCTCGGCGACAACTGCACCAAAGCCGCCTCG	910
F A E L L Y S Q L V S A T N C T K A A S	
TCCTTCGCTTGCTCGAAGCTGTGACGCTGCGGCGCTCGCTGCGGCGGGCGTGAAGAAC	970
S F A C L E A V D A A A L A A A G V K N	
TCGGCGGCGTTCCCGTTCCGGTTTTGGTCGTATGTCCCGGTCGTCGACGGGACCTTCTTG	1030
S A A F P F G F W S Y V P V V D G T F L	

FIG. 8A

ACTGAGCGCGCGTCGCTCCTTCTCGCCAAGGGCAAGAAGAACCTCAATGGCAACCTCTTC	1090
T E R A S L L L A K G K K N L N G N L F	
ACCGGGATCAACAACCTCGACGAAGGATTCATATTCAGTACGCGCCACTATTCAGAACGAC	1150
T G I N N L D E G F I F T D A T I Q N D	
ACGATCAGCGACCGAGTCGCAGCGCGTCTCCAGTTCGACCGCCTCCTCGCCGGCCTCTTC	1210
T I S D Q S Q R V S Q F D R L L A G L F	
CCCTACATCACCTCGGAGGAGCGCCAGGCCGTCGCGAAGCAGTACCCGATCTCCGACGCG	1270
P Y I T S E E R Q A V A K Q Y P I S D A	
CCGTCAAAGGGCAACACCTTCTCTCGCATCTCGGCCGTCATCGCGGACTCGACCTTCGTC	1330
P S K G N T F S R I S A V I A D S T F V	
TGCCCCGACCTACTGGACCGCCGAGGCGTTCGGCTCGTCCGCCCCACAAGGGCCTCTTCGAC	1390
C P T Y W T A E A F G S S A H K G L F D	
TACGCGCCGGCTCACCACGCGACCGACAACCTCGTACTACATCGGCTCCATCTGGAACGGC	1450
Y A P A H H A T D N S Y Y I G S I W N G	
AAGAAGTCGGTCTCGTCCGTCCAGTCCTTCGACGGCGCGCTCGGCGGCTTCATCGAGACG	1510
K K S V S S V Q S F D G A L G G F I E T	
TTCAACCCGAACAACAACGCTGCCAACAAGACCATCAACCCTTACTGGCCGACGTTTCGAC	1570
F N P N N N A A N K T I N P Y W P T F D	
TCGGGCAAGCAGCTCCTCTTCAACACGACGACGAGGGACACCCTCTCTCCCGCCGACCCG	1630
S G K Q L L F N T T T R D T L S P A D P	
CGCATCGTTGAGACTTCAAGCTTGACCGACTTTGGCACGAGCCAGAAGACCAAGTGCGAC	1690
R I V E T S S L T D F G T S Q K T K C D	
TTCTGGCGTGCGTCAATCTCGGTGAACGCGGGTCTC	1726
F W R G S I S V N A G L	

FIG. 8B

CTGCGGCGCTCGCTGCGGCGGGCGTGAAGAACTCGGCGGCGTTCCCGTTCCGGGTTTGGT 1200
A A L A A A G V K N S A A F P F G F W S

CGTATGTCCCGGTCGTGACGGGACCTTCTTGACTGAGCGCGCGTCGCTCCTTCTCGCCA 1260
Y V P V V D G T F L T E R A S L L L A K

-----] [---
AGGGCAAGAAGAACCTCAATGGCGTGCGTGCGGAGCTTTCGAGTGCTTCAGGATCTCGCT 1320
G K K N L N G

-----] [---
GACACTGTGACCGGCTCGCAGAACCTCTTCACCGGGATCAACAACCTCGACGAAGATGA 1380
N L F T G I N N L D E G

-----] [---
GTTCCCGTCGACGGCTCTGTTCCGCCAGCGAGACTGACTTGTTCTTTTGC GAAGATTACG 1440

ATTCATATTCAGTACGCCACTATTCAGAACGACACGATCAGCGACCAGTTCGACGCGCT 1500
F I F T D A T I Q N D T I S D Q S Q R V

CTCCAGTTCGACCGCCTCCTCGCCGGCCTTCTCCCTACATCACCTCGGAGGAGCGCCA 1560
S Q F D R L L A G L F P Y I T S E E R Q

GGCCGTGCGGAAGCAGTACCCGATCTCCGACGCGCCGTCAAAGGGCAACACCTTCTCTCG 1620
A V A K Q Y P I S D A P S K G N T F S R

-----] [---
CATCTCGGCCGTCATCGCGGACTCGACCTTCGTGTGCGTTCCCCGTCGTCTTCTCCGAGT 1680
I S A V I A D S T F V

-----] [---
ATTCCGCTGACTTCCCGCTTGCCCCGAGCTGCCCGACCTACTGGACCGCCGAGGCGTTTCG 1740
C P T Y W T A E A F G

GCTCGTCCGCCCCACAAGGGCCTCTTCGACTACGCGCCGGCTCACCACGCGACCGACA ACT 1800
S S A H K G L F D Y A P A H H A T D N S

CGTACTACATCGGCTCCATCTGGAACGGCAAGAAGTCGGTCTCGTCCGTCCAGTCCTTCG 1860
Y Y I G S I W N G K K S V S S V Q S F D

ACGGCGCGCTCGGCGGCTTCATCGAGACGTTCAACCCGAACAACGCTGCCAACAAGA 1920
G A L G G F I E T F N P N N N A A N K T

CCATCAACCCTTACTGGCCGACGTTTCGACTCGGGCAAGCAGCTCCTCTTCAACACGACGA 1980
I N P Y W P T F D S G K Q L L F N T T T

CGAGGGACACCCTCTCTCCCGCCGACCCGCGCATCGTTGAGACTTCAAGCTTGACCGACT 2040
R D T L S P A D P R I V E T S S L T D F

TTGGCAGGAGCCAGAAGACCAAGTGC GACTTCTGGCGTGGGTCAATCTCGGTGAACGCGG 2100
G T S Q K T K C D F W R G S I S V N A G

GTCTCTAGGCGTCTTTCCTTCCGACTTCCTTCGTTCTTTCGTTGTTTATTCTTGCAGTTC 2160
L *

CGTTGTATCGGCCATTTCGTGCGTGTAGCTCACTCGAGTATAGACGTTGGCAAGTGCGAAA 2220

FIG. 9B

TRN 2-1738 RHODOSPORIDIUM ESTERASE cDNA\$

↑Translation Start ↑Mature Peptide
MLLNLF¹TLASLAATLQLAFASPTSLVRR²TNPNEPPPVDLGYARYQGYLNETAGLYWWRG
IRYASAQRFQAPQTPATHKAVRNATEYGPICWPASEGTNTTKGLPPPSNSSSSAPQKQAS
EDCLFLNVVAPAGSCEGDNLPVLVYIHGGGYAFGDASTGSDFAAFTKHTGTKMVVVNLQY
RLGSFGFLAGQAMKDYGV³TNAGLLDQQFALQWVQQHVSKFGGNPDHVTIWGESAGAGSVM
NQIIANGGNTVKALGLKKPLFHAAIGSSVFLPYQAKYN⁴SPFAELLYSQLVSATNCTKAAS
SFACLEAVDAAALAAAGVKNSAAFPFGFWSYVPVVDGTFILTERASLLAKGKKNLNGNLF
TGINNLDEGFIFTDATIQNDTISDQSQRVSQFDRLLAGLFPYITSEERQAVAKQYPISDA
PSKGNTFSRISAVIADSTFVCPTYWTAEAFGSSAHKGLFDYAPAHHATDNSYYIGSIWNG
KKS⁵VSSVQSFDGALGGFIETFNPN⁶NNNAANKTINPYWPTFDSGKQLLFNTTTTRDTLSPADP
RIVETSSLTDFGTSQKTKCDFWRGSISVNAGL*

FIG. 10

Amino acid composition from 1 to 572
 TRN 2-1738 RHODOSPORIDIUM ESTERASE cDNA

	Total	Percent
A	67	11.7
C	7	1.2
D	25	4.4
E	16	2.8
F	35	6.1
G	49	8.6
H	9	1.6
I	21	3.7
K	25	4.4
L	48	8.4
M	4	0.7
N	35	6.1
P	31	5.4
Q	26	4.5
R	16	2.8
S	52	9.1
T	43	7.5
V	32	5.6
W	10	1.7
Y	21	3.7
Acidic	41	7.2
Basic	41	7.2
Charged	82	14.3
Net charge	0	0.0
Hydrophobic	136	23.8
Residues	572	
MW	61334	

FIG. 11